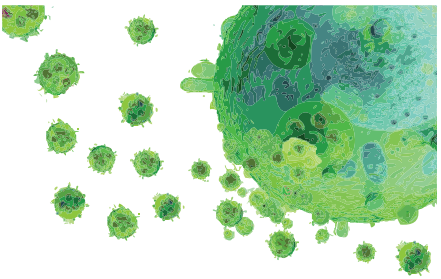

Exosome Antibodies, Array & ELISA Kits

Cat #s EXOAB,EXORAY, EXOEL

User Manual

See page #2 for product storage conditions

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

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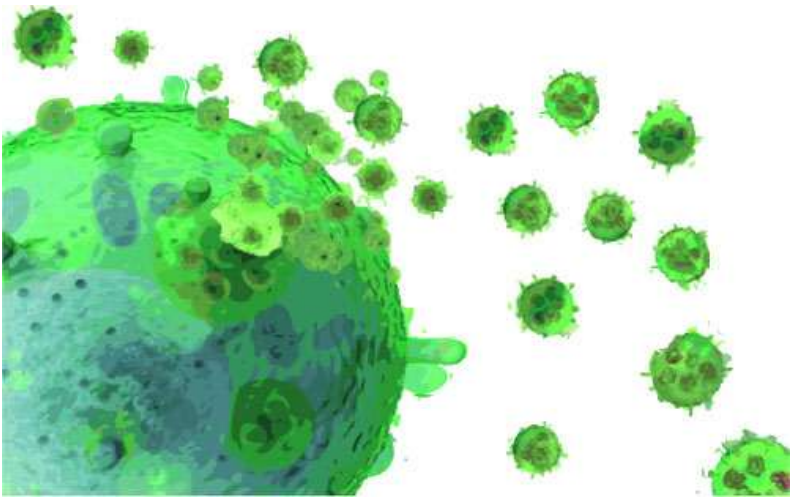


List of Components

EXOAB Series

Exosome Antibodies Components	Amount
Exosome specific primary antibody (CD63,CD9 ,CD81 or Hsp70)	25 µl
Exosome validated secondary antibody (Goat anti-Rabbit HRP)	5 µl

The Exosome Antibodies are shipped in blue ice and should be **stored at +4°C** upon receipt. . Properly stored antibodies are stable for 6 months from the date received. They can be place at **-20°C for long term storage**.

EXORAY Series

Exo-Check Exosome Antibody Arrays
Antibody Membrane Array (PVDF membrane) – 4, 8 or 12 array membranes
Exosome Lysis buffer
Exosome Array Binding buffer
Array Wash buffer
Detection buffer (HRP-conjugated secondary mixture)
Disposable blue forceps to handle membrane arrays

The Exosome Antibody array kits are shipped in blue ice and should be **stored at +4°C** upon receipt. Properly stored antibody array kits are stable for 6 months from the date received.

**** Not supplied:*** HRP developer solution, we recommend the SuperSignal West Femto Chemi-luminescent Substrate, THERMO catalog# 34095.

EXOEL Series

ExoELISA kit Components	Amount
Exosome binding buffer	20 ml
20X Wash buffer	18 ml
Blocking buffer	30 ml
ExoELISA protein standard*	400 µl
Exosome specific primary antibody (CD63,CD9 or CD81)	2 x 25 µl
Exosome validated secondary antibody (Goat anti-Rabbit HRP)	10 µl
ELISA substrate (Super–sensitive TMB)	6 ml
Stop buffer	6 ml
96 well ExoELISA plate (12x8 well strips)	1 plate

The ExoELISA™ kits are shipped in blue ice and should be **stored at +4°C** upon receipt. Properly stored kits are stable for 6 months from the date received.

* ExoELISA protein standards should be **stored at -20°C** upon receipt. We recommend making single-use aliquots to avoid repeated freeze-thawing.

Exosome Antibodies (Cat# EXOAB series)

I. Recommended protocol

-for isolating serum exosomes for Western blotting analysis

1. If frozen, thaw 250 µl serum on ice
2. Centrifuge at 1500 × *g* for 15 minutes to remove cells and cell debris
3. Transfer sample supernatant to a centrifuge tube
4. Combine 250 µl sample + 63 µl **ExoQuick™**
5. Mix well by inversion three times
6. Place at 4ºC for at least 30 minutes
7. Centrifuge at 1500 × *g* for 5 minutes
8. Remove supernatant, keep exosome pellet
9. Centrifuge at 1500 × *g* for 5 minutes to remove all traces of fluid (take great care not to disturb the pellet)

10. Add 200 µl **RIPA buffer**[1] (with appropriate protease inhibitor cocktail added) to exosome pellet and vortex 15 seconds
11. Place at room temperature for 5 minutes (to allow complete lysis)
12. Perform standard Bradford protein assay to determine yield.
13. Add **Laemmli buffer**[2] (with Beta-mercaptoethanol) and heat at 95ºC for 5 minutes.
14. Chilled on ice for 5 minutes before loading onto gel
15. Perform standard SDS-PAGE electrophoresis and Western transfer onto PVDF membrane
16. Block with 5% dry milk in Tris Buffered Saline + 0.05% Tween (TBS-T) for 1 hour
17. Incubate blot overnight at 4°C with **Exosome specific primary antibody** (e.g. CD9**)** at **1:1000 dilution** (5% dry milk in TBS-T)
18. Wash 3X with TBS-T
19. Incubate one hour at room temperature with **Exosome validated secondary antibody** (Goat-Rabbit-HRP) antibody at **1:20,000** dilution (5% dry milk in TBS-T)
20. Wash 3X with TBS-T
21. Incubate blot with chemi-luminescence substrate and visualize on film or other imaging equipment. We recommend the SuperSignal West Femto Chemi-luminescent Substrate, THERMO catalog# 34095.

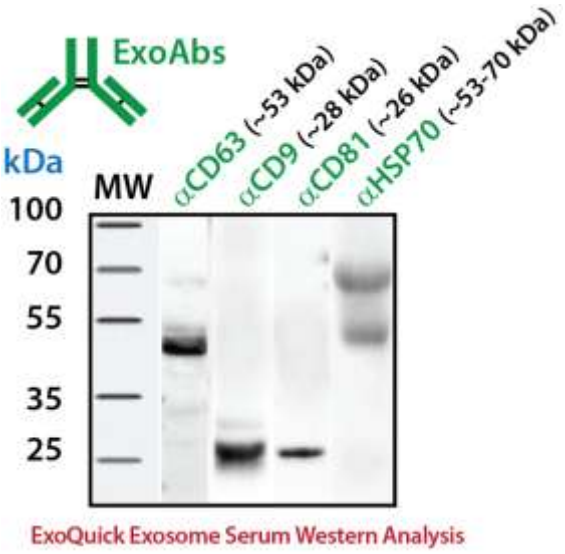
[1] 1X **RIPA buffer** contains:

- 25mM Tris-HCl pH 7.6
- 150mM NaCl
- 1% NP-40
- 1% sodium deoxycholate
- 0.1% SDS

[2] 2X **Laemmli buffer** contains:

- 4% SDS
- 20% glycerol
- 10% 2-mercaptoethanol
- 0.004% bromophenol blue
- 0.125 M Tris-HCl pH 6.8

II. Serum Exosome Western Data



Western blot analysis of serum exosomes using the recommended protocol. 20 µg of serum exosome proteins were loaded into each lane. All **Exosome specific primary antibodies** were used at **1:1,000** dilution and **Exosome validated secondary antibody** was used at **1:20,000** dilution. We recommend the SuperSignal West Femto Chemi-luminescent Substrate, THERMO catalog# 34095.

Exosome Antibody Arrays (Cat# EXORAY series)

The Exo-Check antibody array has 12 pre-printed spots and features 8 antibodies for known exosome markers (CD63, CD81, ALIX, FLOT1, ICAM1, EpCam, ANXA5 and TSG101) and a GM130 cis-Golgi marker to monitor any cellular contamination in your exosome isolations. The array is compatible with ultracentrifugation and ExoQuick methods. The positive control spot is derived from human serum exosome proteins and are printed directly onto the membrane. Your exosome preparations are lysed and then incubated with the array for the pre-printed antibodies to capture their respective exosome proteins. The GM130 antibody spot on each array is included to help identify and cellular contamination in your exosome preparations. The kits come complete with a secondary detection mixture conjugated to HRP. The arrays are semi-quantitative and can be used to evaluate relative abundance of certain exosome protein

markers from a set of given samples. The orientation of the Array is identified as using the upper left hand corner that is notched.

I. Protocol for Exo-Check Antibody Arrays

Sample preparation and antibody array capture

1. Precipitate sample exosomes from serum or culture media using ExoQuick or ExoQuick-TC (also compatible with exosome pellets from ultracentrifugation) to obtain an exosome pellet. We suggest when using ExoQuick, start with 500 ul serum sample 300 ul PBS. For culture media, we suggest starting with 10 ml media and then resuspend exosome pellet with 300 ul PBS.
2. Use a small volume of resuspended exosomes and perform a protein assay to determine the recovery.
3. Add 300-500 ug exosome proteins with 600 ul Exosome Lysis buffer.
4. Vortex for 15 seconds to prepare exosome protein lysate mixture.
5. In a fresh 15 mL Falcon tube, combine the 600 ul exosome lysate with 9.4 mL Exosome Array Binding buffer and mix by inverting 3 times.
6. In a small, clean tray, briefly wet the antibody array in 5 mL distilled water for 2 minutes at room temperature.
7. Decant the water from the membranes. Pipette the 10 mL Exosome lysate / binding mixture to one Exo-Check antibody membrane array. Place the membrane “face-up” by positioning the membrane such that the upper left notched corner can be seen in the left hand side on top.
8. Incubate the tray/membrane mixture shaking at 2-8°C overnight on a shaker or a rocker.

Antibody array washing and signal detection

1. Decant the Exosome lysate binding mixture from the membrane carefully.
2. Add 10 mL Array wash buffer (made into 1X from 20X stock) and shake gently for 5 minutes at room temperature.
3. Decant the Array wash buffer.
4. Add 10 mL Detection buffer to Array membrane and gently shake at room temperature for 2 hours.
5. Prepare the Developer mixture (not included) about 5 minutes before the end of Step #5 by combining 1 mL solution A with 1 mL solution B together. We recommend the SuperSignal West Femto Chemi-luminescent Substrate, THERMO catalog# 34095.
6. Decant the Detection buffer
7. Add 10 mL Array wash buffer (made into 1X from 20X stock) and shake gently for 5 minutes at room temperature.
8. Decant the Array wash buffer, repate twice for a total of three washes.

9. Add the 2 mL Developer solution mixture from Step 5 directly onto the Array membrane so that it is completely covered.
10. Incubate at room temperature for 2 minutes.
11. Decant the developer solution and image the Array signals.
    a. If you use a Bio-Rad ChemiDoc XRS Imager System (or similar), expose 4-10 seconds.
    b. Expose Array membrane to Kodak XR film for 1-3 minutes and develop in a film developer machine**.**

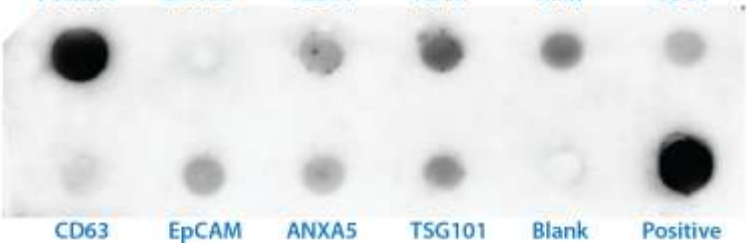
II. Sample Data for Exo-Check Antibody Arrays

What to expect

The positive control signals should provide a bright signal and this will indicate that all of the detection reagents are working properly. The various exosome antibody spots will provide signals of varying degree, depending upon the source of the isolated exosomes. The Blank spot should not have any signal and this serves as a background control. The GM130 control is to evaluate cellular contamination (if any) in your exosome preparations. The data below are from 300 ug of exosome proteins isolated using ExoQuick-TC from human HT1080 lung sarcoma cell line media.

Array format

	1	2	3	4	5	6
A	Positive	GM130	FLOT-1	ICAM	ALIX	CD81
B	CD63	EpCAM	ANXA5	TSG101	Blank	Positive



Information about the exosome protein targets

Location	ID	Gene Name	Exocarta Link/Comments
A1	Positive	Positive control for HRP detection	
A2	Blank	Negative control for background	No antibody loaded, test for background signals
A3	FLOT1	Flotillin 1	http://exocarta.org/gene_summary?gene_id=10211
A4	ICAM1	Intercellular adhesion molecule 1	http://exocarta.org/gene_summary?gene_id=3383
A5	ALIX	PDCD6IP; AIP1; ALIX; Programmed cell death 6-interacting protein; ALG-2-interacting protein 1; Hp95	http://exocarta.org/gene_summary?gene_id=10571
A6	CD81	IGSF8, Immunoglobulin superFamily, member 8	http://exocarta.org/gene_summary?gene_id=93185
B1	CD63	CD63, LAMP-3	http://exocarta.org/gene_summary?gene_id=967
B2	EpCam	Epithelial cell adhesion molecule	http://exocarta.org/gene_summary?gene_id=4072
B3	ANXA5	Annexin A5	http://exocarta.org/gene_summary?gene_id=308
B4	TSG101	Tumor susceptibility gene 101	http://exocarta.org/gene_summary?gene_id=7251
B5	GM130	cis-Golgi matrix protein	Monitor cellular contamination in exosome preps. If this signal is positive, then there are possible cellular debris contaminants present.
B6	Positive	Positive control for HRP detection	

ExoELISA™ - (Cat# EXOEL series)

I. ExoELISA principle

The ExoELISA kit is designed as a direct Enzyme-Linked ImmunoSorbent Assay (ELISA). The lysed exosome particles and their proteins are directly immobilized onto the wells of the microtiter plate. After binding, the wells are coated with a block agent to prevent non-specific binding of the primary detection antibody. The detection (primary) antibody is added to the wells for binding to a specific antigen (e.g. CD63) protein present in the exosome lysates. A Horseradish Peroxidase enzyme linked secondary antibody (Goat anti-Rabbit) is used for signal amplification and to increase assay sensitivity. A colorimetric substrate (Extra-sensitive TMB) is used for the assay read-out. The accumulation of the colored product is proportional to the specific antigen present in each well. The results are quantitated by a microtiter plate reader at 450 nm absorbance.

II. Equipment to be supplied by user

1. Microtiter plate sealing film/cover
2. 37°C incubator
3. Microtiter plate shaker
4. Microtiter plate spectrophotometer with 450 nm absorbance capability
5. Multichannel pipets (recommended)

III. Protocol

A. Exosomes precipitation with ExoQuick/ExoQuick-TC

For simple and quick isolation of exosomes from serum, we recommend using the ExoQuick precipitation solution (Catalog# EXOQ5A-1 or EXOQ20A-1) and the ExoQuick-TC for isolation of exosomes from tissue culture media and urine samples (EXOTC10A-1 or EXOTC50A-1).

1. If frozen, thaw sample on ice
2. Centrifuge at 3000 × g for 15 minutes to remove cells and cell debris.
3. Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick or ExoQuick-TC.

Incubation time	Bio-fluid	Sample volume	ExoQuick volume	ExoQuick-TC voulme
30 minutes	Serum	250 µl	63 µl	-
Overnight	Ascites fluid	250 µl	63 µl	-
Overnight	Culture Media	5 ml	-	1 ml
Overnight	Urine	5 ml	-	1 ml
Overnight	Spinal fluid	5 ml	-	1 ml

4. Mix well by inversion three times
5. Place at 4 ºC from 30 minutes to overnight according to table.
6. Centrifuge at 1500 × *g* for 30 minutes
7. Remove supernatant, keep exosome pellet
8. Centrifuge at 1500 × *g* for 5 minutes to remove all traces of fluid (take great care not to disturb the pellet)
9. Add 200 µl **Exosome Binding buffer** to exosome pellet and vortex 15 seconds

10. Incubate at 37 ºC temperature for 20 minutes to liberate exosome proteins
11. Centrifuge at 1500 × *g* for 5 minutes to remove all residual precipitation solution
12. Transfer supernatant to new centrifuge tube on ice
13. Exosome protein is now ready for immobilization onto micro-titer plate

Proceed to section - **C. Exosome protein standard curve**

B. Exosomes isolated with ultracentrifugation method

1. Performed exosome isolation by the user's preferred ultra-centrifugation protocol
2. Carefully remove all traces of supernatant, keep exosome pellet
3. Resuspend exosome pellet in 200 µl of **Exosome binding buffer** and vortex 15 seconds
4. Incubate at room temperature for 10 minutes and keep on ice.
5. Exosome protein is now ready for immobilization onto micro-titer plate

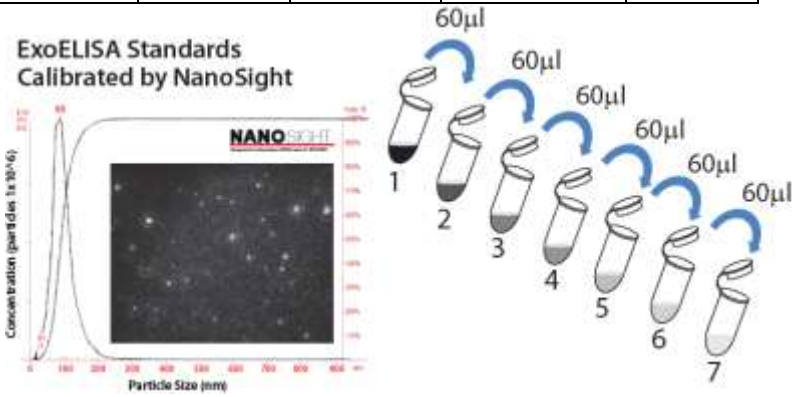
Proceed to section - **C. Exosome protein standard curve**

C. Exosome protein standard curve

A standard curve should be prepared each time the assay is performed

1. Thaw **ExoELISA protein standard** on ice
2. Dilute **ExoELISA protein standard** by performing serial dilutions with **Exosome Binding buffer** in microcentrifuge tubes
3. Suggested dilutions for standard curve:

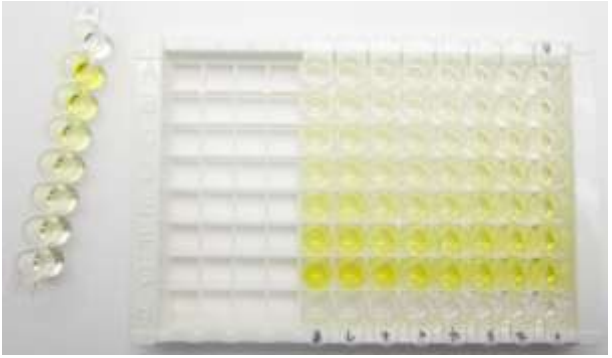
Tube	# Exosomes	Dilution factor	ExoELISA protein standard	Exosome binding buffer
0	0	Blank	-	60
1	$1.35x10^{10}$	1	60 µl	-
2	$6.75x10^{9}$	1:2	60 µl	60 µl
3	$3.37x10^{9}$	1:4	60 µl	60 µl
4	$1.68x10^{9}$	1:8	60 µl	60 µl
5	$8.44x10^{8}$	1:16	60 µl	60 µl
6	$4.21x10^{8}$	1:32	60 µl	60 µl
7	$2.10x10^{8}$	1:64	60 µl	60 µl



D. ELISA procedures

Before starting

1. The ExoELISA micro-titer plate is provided in a convenient 8 well X 12 strip format. We recommend using at least one strip for the standard curve and additional strips depending on the number of samples tested. Unused 8-well strips can be removed and stored at room temperature for later use.
2. Make sure to warm the **Super-sensitive TMB ELISA** substrate to room temperature before adding to the ELISA plate wells in step #10.



3. Dilute stock **20X Washing buffer** into **1X working Wash buffer** with purified water (each 8-well strip requires approximately 10 ml of 1X Washing solution)

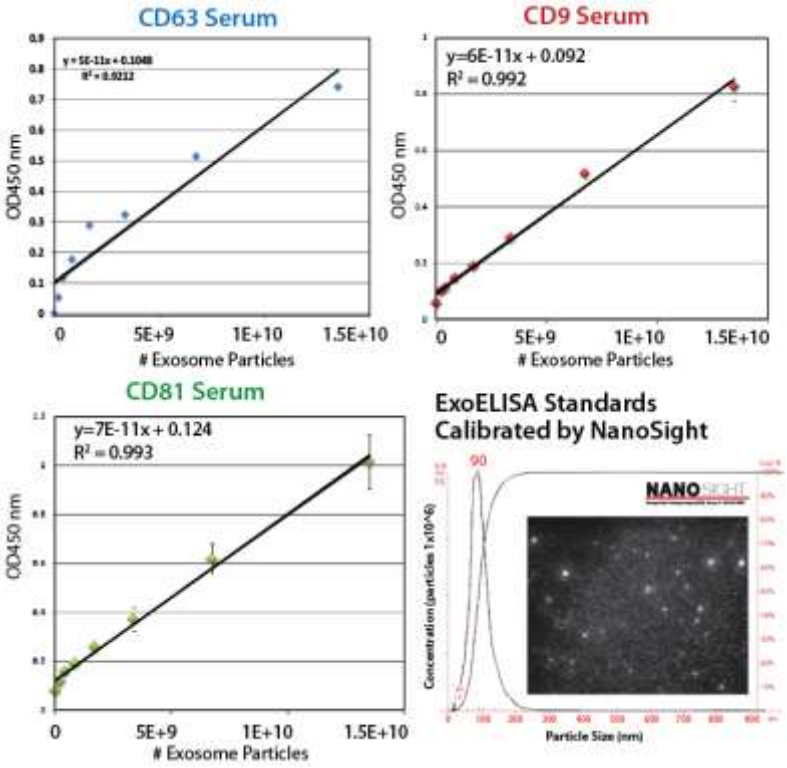
ELISA assay

1. Add 50 µl of prepared protein standards and exosome protein sample to the appropriate well of the micro-titer plate
2. Cover plate with sealing film/cover
3. Incubate the plate at 37ºC from 2 hours to overnight (recommended)
4. After incubation step, invert the plate to empty all contents.
5. Wash the plate 3 times for 5 minutes each with 100 µl **1X Wash buffer**
   - A micro-titer plate shaker is recommend for all subsequent the washing and incubation steps
   - Residual liquid should be removed by hard-tapping the plate on fresh paper towels, while taking care not to let the wells dry out completely
6. Dilute **Exosome specific primary antibody (CD63, CD9 or CD81)** 1:100 in 1X blocking buffer and add 50 µl of to each well and incubate at room temperature for 1 hour with shaking
7. Wash the plate 3 times for 5 minutes each with 100 µl **1X Wash buffer**
8. Dilute **Exosome validated secondary antibody** 1:5,000 1X blocking buffer and add 50 µl to each well and incubate at room temperature for 1 hour with shaking
9. Wash the plate 3 times for 5 minutes each with 100 µl **1X Wash buffer**
10. Add 50 µl of **Super-sensitive TMB ELISA** substrate and incubate at room temperature for 15 to 45 minutes with shaking
    -15 to 45 minutes substrate incubation time is optimized for the recommended exosome protein standard curve

- Further optimization maybe required by the user for individual sample.

11. Add 50 µl of **Stop buffer** to provide a fixed endpoint for the assay
    *- Note that the initial color of a positive sample is blue and the color changes to yellow when **Stop buffer** is added*
12. Quantitate results with a spectrophotometric plate reader at 450 nm absorbance

E. Sample Data



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VI. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

System Biosciences (SBI)
265 North Whisman Road.

Mountain View, CA 94043

Phone: (650) 968-2200
(888) 266-5066 (Toll Free)

Fax: (650) 968-2277

E-mail:

General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

VII. Licensing and Warranty Statement

Limited Use License

Use of the ExoAB antibodies, Exo-Check antibody Arrays and ExoELISA Kits (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.
SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

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